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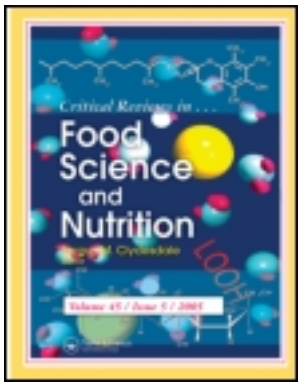

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Francina R. Baldrick [a], Jayne V. Woodside [a], J. Stuart Elborn [b], Ian S. Young [a] & Michelle C. McKinley [a]

[a] Nutrition and Metabolism Group, Centre for Public Health, Queen's University Belfast, UK

[b] Respiratory Research Group, Centre for Infection and Immunity, Queen's University Belfast, UK

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Biomarkers of Fruit and Vegetable Intake in Human Intervention Studies: A Systematic Review

FRANCINA R. BALDRICK,[1] JAYNE V. WOODSIDE,[1] J. STUART ELBORN,[2] IAN S. YOUNG,[1] and MICHELLE C. MCKINLEY[1]

[1]Nutrition and Metabolism Group, Centre for Public Health, Queen's University Belfast, UK
[2]Respiratory Research Group, Centre for Infection and Immunity, Queen's University Belfast, UK

Observational evidence consistently shows that consumption of a diet rich in fruit and vegetables may offer protection against diseases such as cardiovascular disease and cancer. Assessment of dietary intake is complex and prone to many sources of error. More objective biomarkers of fruit and vegetable intake are therefore of interest. The aim of this review is to examine the usefulness of the main biomarkers of fruit and vegetable intake to act as objective indicators of compliance in dietary intervention studies. A comprehensive search of the literature was conducted using six databases. Suitable papers were selected and relevant data extracted. The papers were categorized into 3 sub-groups: whole diet interventions; mixed fruit and vegetable interventions; and studies involving individual varieties of fruits or vegetables. Ninety-six studies were included in the review. Overall, the most commonly measured, and most consistently responsive, biomarkers were the carotenoids and vitamin C. Based on the results of this systematic review, it remains prudent to measure a panel of biomarkers in fruit and vegetable intervention studies. The only possible exception to this is "fruit only" intervention studies where assessment of vitamin C alone may suffice.

Keywords carotenoids, flavonoids, urinary potassium, vitamin C, glucosinolates, dietary intake

INTRODUCTION

There is considerable observational evidence to suggest that a diet rich in fruit and vegetables may offer protection against non-communicable diseases such as cancer, stroke, and coronary heart disease (CHD) (Joshipura et al., 2001; Hu, 2003; Riboli and Norat, 2003; Dauchet et al., 2006; He et al., 2006). It is not yet established whether it is consumption of fruit or vegetables in general, individual varieties of fruits or vegetables, or specific nutritional components within fruit and vegetables which are accountable for these benefits (Jansen et al., 2004). Further elucidation of this diet-disease relationship, for example, via randomized controlled trials, and assessment of population intakes in dietary surveys requires accurate and reliable assessment of fruit and vegetable intake. Traditional methods of assessing fruit and vegetable intake include the use of dietary assessment tools such as food diaries, food frequency questionnaires, or 24-hour recalls (Tucker et al., 1999; Kirsh et al., 2007; Mikkelsen et al., 2007). However, it is well recognized that self-reported intake can often be inaccurate (Horner et al., 2002) due, for example, to reliance on a participant's memory, an inability of some methods to account for day-to-day variation in intake, or the fact that respondents tend to change their usual eating patterns in order to simplify record keeping and/or to impress the investigator (Lee and Niemen, 2003; Livingstone and Black, 2003). These problems can be further compounded by coding and data entry errors (Deharveng et al., 1999; Lee and Niemen, 2003).

Given the difficulties associated with dietary intake methodology outlined above, more objective and accurate indices of fruit and vegetable intake, such as nutritional biomarkers, are therefore of interest. Such biomarkers of intake need to be able to discriminate between differences in intakes (Hunter, 1990; Crews et al., 2001), should be non-invasive or minimally invasive (Crews et al., 2001; Field et al., 2001), reproducible, easily measured (Stockley, 2007), and highly responsive to the intervention being carried out (Crews et al., 2001). Plasma and serum biomarkers have been explored as potentially

Address correspondence to Michelle McKinley, Nutrition and Metabolism Group, Centre for Public Health, School of Medicine, Dentistry and Biomedical Sciences, 1st Floor, Institute of Clinical Science B, Grosvenor Road, Belfast BT12 6BJ, Northern Ireland, U.K. Tel.: +44 28 90632685; Fax: +44 28 90635900. E-mail: m.mckinley@qub.ac.uk

useful indicators of fruit and vegetable intake. For instance, fruit and vegetables are the primary source of carotenoids in the diet, and as carotenoids cannot be synthesized by humans, they are considered to be good candidate biomarkers of intake (Jansen et al., 2004). Six carotenoids (α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, lycopene, and lutein) are found in appreciable amounts in human serum (Crews et al., 2001; Al-Delaimy et al., 2005). Other candidate biomarkers of fruit and vegetable intake include antioxidant vitamins such as vitamin C (Dehghan et al., 2007; Padayatty and Levine, 2008) and flavonoids (Mennen et al., 2006), including quercetin (McAnlis et al., 1999). Some of these compounds have been reliably associated with a particular fruit or vegetable, or a class of fruit or vegetables in observational studies, but less successfully with total fruit or vegetable consumption (Campbell et al., 1994; Bingham et al., 1997; Drewnowski et al., 1997; Jansen et al., 2004; Padayatty and Levine, 2008). This is almost certainly owing to the complexity of fruit or vegetables and the large number of bioactive compounds present, but also potentially because of other dietary sources of these compounds.

The aim of this systematic review is to examine the utility of the main biomarkers of fruit and vegetable intake to act as objective indicators of compliance in dietary intervention studies. This review will, therefore, only examine data from human intervention trials.

METHODS

Selection Criteria

The search strategy detailed below was devised in order to examine data from intervention studies that aimed to increase fruit and vegetable intake, by whatever means (e.g., dietary advice, provision of fruit and vegetables, or provision of whole diet), as the sole focus of an intervention or as part of a wider dietary intervention.

Search Strategy

The search strategy was developed in PUBMED and adapted for EMBASE, Medline, Science Citation Index (SCI), Cumulative Index to Nursing and Allied Health Literature (CINAHL) and the Cochrane Library. The search was limited to intervention studies in adults published in the English-language. The search terms used were "fruit" and "vegetables" combined with "compliance," which was then combined with "intervention" or "trial." These terms were then combined with "biomarkers," "biological markers," "antioxidants," "carotenoids," "vitamin A," "vitamin C," "ascorbate," "ascorbic acid," "alpha-carotene," "beta-carotene," "beta-cryptoxanthin," "zeaxanthin," "lycopene," "lutein," "urinary potassium," "glucosinolates" and "flavonoids." In addition to the databases above, the bibliographies of retrieved articles were also reviewed to obtain additional citations. The search was first conducted on 23/04/2007 and last updated on 23/04/2009.

Selection of Documents

Articles were rejected on initial screening if the reviewer could determine from the title that the study was not a fruit and vegetable intervention study. Abstracts were then obtained for all other studies and examined against the selection criteria. When a paper could not be included or excluded with certainty from the abstract, the full text of the article was acquired for further evaluation. The suitability of each paper was assessed by one reviewer (FB) and checked by a second (MMcK). The study selection procedure is summarized in Fig. 1.[1]

Acute studies, where the intervention involved a single ingestion of the intervention food(s) were excluded, as were tomato-based studies as this has been covered extensively in the literature. A number of studies were excluded when it became clear on examination of the full paper that participants were not given specific advice to increase fruit and vegetable intake as part of the intervention. Studies were also excluded if the intervention used fruit juice diluted with water rather than pure fruit juice as this is not classified as contributing to fruit and vegetable intake (van den Berg et al., 2001; Bub et al., 2003; Moller et al., 2004). If several papers reported results on the same study, but using datasets with different numbers of participants, the publication reporting the largest number of participants was used (Pierce et al., 1997; 2006; Rock et al., 1997; McEligot et al., 1999). If multiple publications of the same data set existed, the original paper or the paper reporting the most complete data set was used (Bub et al., 2000; Broekmans et al., 2001; Pierce et al., 2002; Steptoe et al. 2004; Watzl et al., 2005).

Papers were then categorized into 3 sub-groups: (i) whole diet intervention studies (advice to increase fruit and vegetable intake was one component of a whole diet approach); (ii) mixed fruit and vegetable studies (intervention involved administration of more than one type of fruit or vegetable); and (iii) individual fruit and vegetable intervention studies (study groups increased consumption of one specific type of fruit or vegetable). The mixed fruit and vegetable studies were further sub-divided into 2 groups: a) food provision studies (fruit and vegetables or whole diet provided), and b) studies using counselling methods to increase consumption. The individual fruit and vegetable studies were also further sub-divided into 2 groups: a) fruit and fruit juice studies, and b) vegetable and vegetable juice studies.

Data Abstracted

Three types of variables were extracted from each study:

i. Dietary intervention—nature of the intervention and control diets and their implementation strategy, the study type (e.g.,

[1]95 papers were included in this review (study selection summarized in Fig. 1 next page). However, one paper reported data for three different studies: one was excluded as it was a single dose acute study; one study is included in the "individual fruit and vegetable studies" section; and one study is included in the "mixed fruit and vegetable studies" section. Hence data for a total of 96 studies is reported in the review.

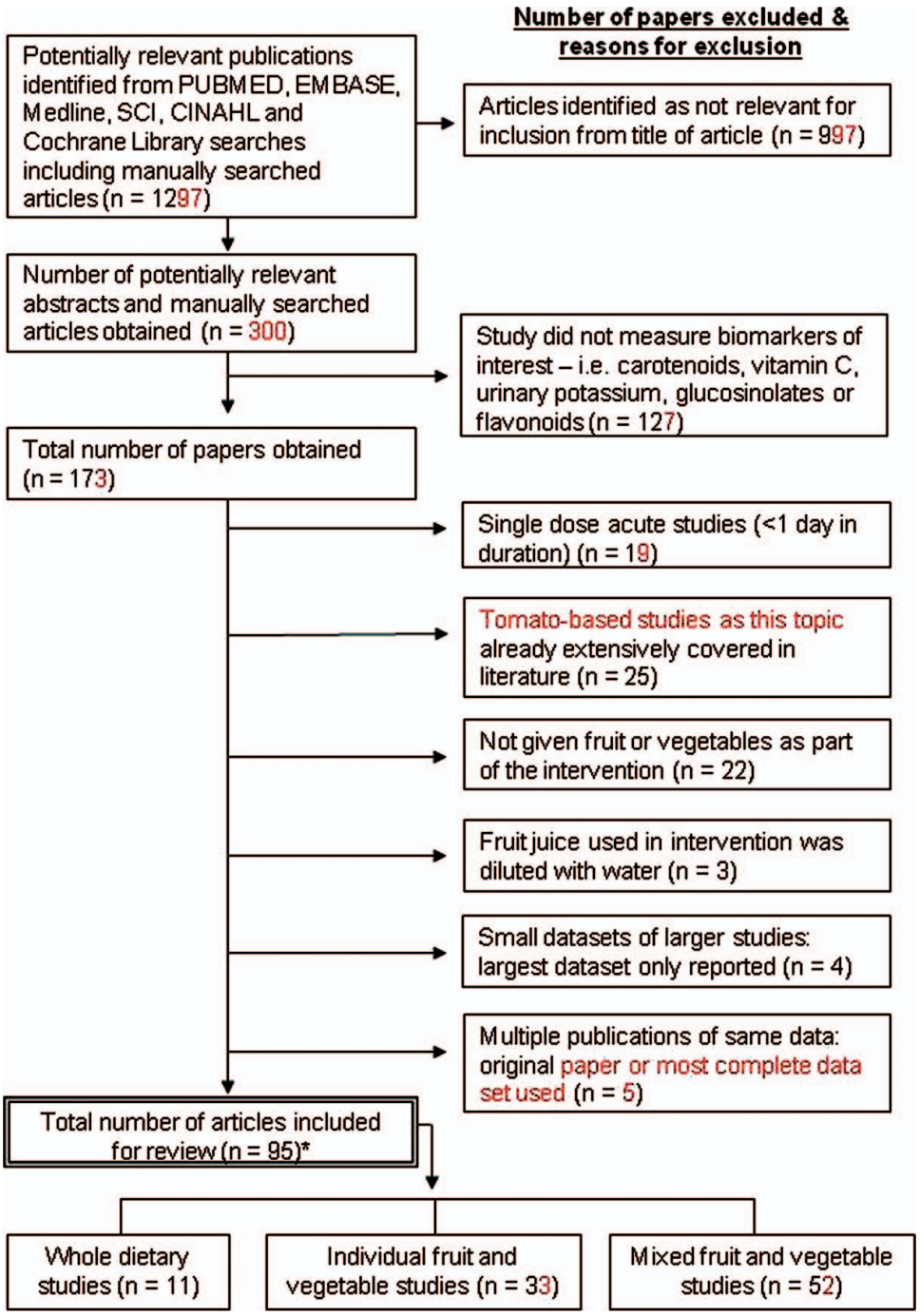

Figure 1 Summary of study selection procedure. (color figure available online.)

food provision; parallel or crossover study), and the duration of dietary intervention (days/years).

ii. Participants studied—type and number of participants enrolled (e.g., healthy volunteers, breast cancer or myocardial infarction survivors).

iii. Outcome variables—change in fruit and vegetable intake (reported or recommended) in response to the intervention and change in plasma or serum levels of α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, lycopene, and vitamin C. Flavonoids, urinary potassium, glucosinolates, and total carotenoids have been dealt with in the footnotes of the results tables as only a limited number of studies have examined these. Data on α-tocopherol, γ-tocopherol, and retinol was not extracted and is not discussed in this review. Data on tocopherols is frequently reported in fruit and vegetable interventions, as these compounds are commonly also measured by laboratory methods that assess carotenoid concentrations; however, their main dietary sources are not

fruit and vegetables and most studies report no changes in serum levels. Retinol is also not discussed as the bioconversion of provitamin A carotenoids in plant foods to retinol is of a much smaller magnitude than previously thought (West et al., 2002), hence it is an unreliable biomarker.

RESULTS

Biomarker responses to the various interventions (whole diet intervention studies; mixed fruit and vegetable studies—food provision or counselling studies; and individual fruit and vegetable intervention studies—fruit and fruit juice studies, and vegetable and vegetable juice studies) are summarized in Tables 1–5 (Note: various flavonoids, total carotenoids, glucosinolates, and urinary potassium were measured infrequently and so data is not presented in the tables, however, the table footnotes do indicate which studies measured these biomarkers and the results are summarized in the text). Data presented in these tables represents within group change between baseline and post-intervention, unless otherwise stated.

Whole Diet Interventions

As indicated in Table 1, the search strategy identified 11 studies that employed a whole diet approach to dietary change, one component of which was advice to increase fruit and vegetable intake. Of the 11 suitable whole diet studies identified, only two used a controlled feeding approach (Miller et al., 2005b; Turban et al., 2008); the others used a variety of techniques such as telephone, individual, and group counselling and information provision in the form of booklets, and recipes and menu cycles to encourage dietary change (de Lorgeril et al., 1998; Lanza et al., 2001; Shike et al., 2002; Pierce et al., 2004; Vincent-Baudry et al., 2005; Prentice et al., 2006; Wanke et al., 2007; Newman et al., 2008; Parsons et al., 2008).

In summary, the number of biomarkers measured in these whole diet studies varied from one to seven. A significant increase in the biomarkers of interest was reported in 8 out of the 11 (73%) studies included in this section. In terms of the response of individual biomarkers, α- and β-carotene increased significantly in three out of seven (43%) and four out of seven (57%) studies respectively, lycopene increased in two out of seven (29%) studies, β-cryptoxanthin in one out of seven (14%) studies, lutein in three out of four (75%) studies, zeaxanthin in one out of two (50%) studies, lutein/zeaxanthin together in two out of three (67%) studies, and vitamin C in one out of one study (100%). Total carotenoids increased significantly in two (Lanza et al., 2001; Parsons et al., 2008) out of five (40%) studies reporting such data (data not shown; Lanza et al., 2001; Prentice et al., 2006; Wanke et al., 2007; Newman et al., 2008; Parsons et al., 2008). Urinary potassium was only measured in one study (Turban et al., 2008; data not shown), which reported a significant increase.

Mixed Fruit and Vegetable Studies

The protocol in the fifty-two studies identified for inclusion in this section of the review focused on increasing consumption of a variety of fruit and vegetables. These studies were further sub-divided into studies that used a counselling approach to achieve dietary change (Table 2, n = 16) and food provision studies (Table 3a and 3b, n = 36).

Counselling Studies

As shown in Table 2, 16 studies used a counselling approach to specifically encourage an increase in fruit and vegetable intake. In general, the studies involved encouraging participants to increase their fruit and vegetable intake to at least 5 portions of fruit and vegetables per day in the UK, and to approximately 8–10 portions per day in the USA, reflecting differing dietary guidelines between countries (potatoes are included in the fruit and vegetable classification in the USA but not in the UK).

The number of biomarkers measured in these counselling studies varied from one to eight, and most studies (n = 13; 81%) measured four or more biomarkers. Huxley et al. (2004) who measured 3 flavonoids (quercetin, kaempferol and isorhamnetin; data not shown) and Cartmel et al. (2005) who measured 6 carotenoids, did not detect a significant increase in the biomarkers they measured. However, Huxley et al. (2004), who reported data for quercetin, kaempferol, and isorhamnetin in the Oxford Fruit and Vegetable study, attributed this to the non-specific nature of the intervention and the fact that the timing of the blood sample was not standardized. John et al. (2002) (Table 2), report the main results from the Oxford Fruit and Vegetable study and highlight significant increases in several carotenoids and vitamin C. In terms of individual biomarker responses in the mixed fruit and vegetable counselling studies, α- and β-carotene increased significantly in 12 out of 14 (86%) studies and 13 out of 15 (87%) studies respectively, β-cryptoxanthin in eight out of 12 (67%) studies, lycopene in four out of 12 (33%) studies, vitamin C in six out of ten (60%) studies, lutein in five out of seven (71%) studies, zeaxanthin in one out of five (20%) studies, lutein/zeaxanthin in three out of five (60%) studies. Total carotenoids increased significantly in four (Le Marchand et al., 1994; Smith-Warner et al., 2000; Rock et al., 2001; Bernstein et al., 2002) out of six (67%) studies (Le Marchand et al., 1994; Smith-Warner et al., 2000; Rock et al., 2001; Bernstein et al., 2002; Takashashi et al., 2003; Cartmel et al., 2005; data not shown). Flavonoids (kaempferol, isorhamnetin, and quercetin) were only measured in one study and did not change significantly (Huxley et al., 2004; data not shown), and urinary potassium was measured in two studies but did not change significantly (Steptoe et al., 2003; Takashashi et al., 2003; data not shown).

Table 1 Change in potential biomarkers of fruit and vegetable intake in response to whole diet interventions (advice to increase fruit and vegetable intake was one component of a whole diet approach)

					Biomarker response to intervention							
Study	Intervention	Depletion period	Intervention duration	Increase in F&V intake (servings/d)*	α-carotene	β-carotene	β-cryptoxanthin	Lycopene	Lutein	Zeaxanthin	Lutein + zeaxanthin	Vitamin C
PPT (Lanza et al., 2001),[1,2] USA	LF; HF; High F&V (5–8 servings/d)	No	1 year	Yr1 : 2.7 − 3.0	—	—	—	—	—	—	—	—
			4 years	Yr4 : 3.2 − 4.1	—	—	—	—	—	—	—	—
PPT (Shike et al., 2002), USA	LF; HF; High F&V (5–8 servings/d)	No	4 years	2.6	↑	NSC	NSC	NSC	—	—	↑	—
PPT adherence study (Wanke et al., 2007)[1], USA	LF; HF; High F&V (5–8 servings/d)	No	4 years	1.5 (poor)	—	—	—	—	—	—	—	—
				2.7 (inconsistent)	—	—	—	—	—	—	—	—
				3.3 (super)	—	—	—	—	—	—	—	—
WHEL Study (Pierce et al., 2004), USA	Reduced fat; HF; High F&V (8+ servings/d)	No	1 year	3.9	↑	↑	NSC	↑	—	—	↑	—
DASH-sodium ancillary study (Miller et al., 2005b), USA†	DASH-sodium diet	No	3 months	6 **	↓ †	↑ †	↑ †	NSC	↑ †	↑ †	—	—
DASH cohort study (Turban et al., 2008)[3], USA	i) High F&V diet	No	2 months	i) 4.9**	—	—	—	—	—	—	—	—
	ii) DASH diet			ii) 6 **	—	—	—	—	—	—	—	—
Lyon Trial (de Lorgeril et al., 1998)[1], France	Mediterranean type diet	No	2 months	7.1§	—	—	—	—	—	—	—	↑
Medi-RIVAGE study (Vincent-Baudry et al., 2005), France	Mediterranean diet	No	3 months	1.7	NSC	NSC	NSC	NSC	NSC	NSC	—	—
WHI Study(Prentice et al., 2006), USA	↓ total fat intake	No	1 year	1.5	NR	NR	NR	NR	—	—	NR	—
	F&V (≥5 servings/d) ↑ Grains		3 years	NR	NSC	NSC	NSC	NSC	—	—	NSC	—
Healthy Eating Program subsample study (Newman et al., 2008), USA	High F&V (6–9 servings/d) 3 whole grain &/or bean or legume/d	No	6 months	4.7	NSC	↑	NSC	NSC	↑	—	—	—
MEAL study (Parsons et al., 2008), USA	7 servings vegetables/d 2 servings whole grains/d 1 serving beans/legumes/d	No	6 months	3	↑	↑	NSC	↑	↑	—	—	—

F&V = fruit and vegetables; **LF** = low fat; **HF** = high fiber;

↑ = significant increase in biomarker; ↓ = significant decrease in biomarker; — = not measured; **NR** = not reported; **NSC** = no significant change;

*Increase in intake calculated based on UK Department of Health guidance on portion sizes (1 serving = 80 g fruit/vegetables or 150 mL juice) - data given is reported increase in intake unless otherwise stated;

[1]Study reported between group statistics.

[2]Lanza et al. (2001) and Wanke et al. (2007) only measured total carotenoids (see text for details).

[3]Turban et al. (2008) only measured urinary potassium (see text for details).

As reported in Appel et al. (1997). *NEJM*. **336(16): 1117–1124.

†Estimated from Fig. 5, Miller et al. (2005b). *Atherosclerosis*. **183**: 175–182.

§Reported intake of fruit and vegetables after 1 to 4 years follow-up, as reported in Table 5, de Lorgeril et al. (1994). *Lancet*. **343**: 1454–1459.

Table 2 Change in potential biomarkers of fruit and vegetable intake in response to increased consumption of mixed fruit and vegetables using counselling methods

Study	Intervention	Depletion Period	Intervention duration	Increase in F&V intake (servings/d)*	Biomarker response to intervention							
					α-carotene	β-carotene	β-cryptoxanthin	Lycopene	Lutein	Zeaxanthin	Lutein + zeaxanthin	Vitamin C
Thompson et al. (1999), USA	5 servings F&V/d	No	2 weeks	6.2	↑	↑	↑	↑	↑	—	—	—
Record et al. (2001), Australia	5–7 servings F&V/d and juice	2 weeks – Avoid antioxidants	2 weeks	~ 2.0 – 4.0**	↑	↑	NR	NSC	—	—	↑	↑
Nelson et al. (2003), USA	High carotenoid diet	No	5 weeks	NR#	NSC	↑	↑	↑	↑	↑	—	NSC
Zino et al. (1997)[1], New Zealand	F&V (8 servings/d)	No	2 weeks	4.7§	↑	↑	—	—	—	—	—	↑
			4 weeks		↑	↑	—	—	—	—	—	↑
			6 weeks		↑	↑	—	—	—	—	—	↑
			8 weeks		↑	↑	—	—	—	—	—	↑
Le Marchand et al. (1994), USA	F&V (8 servings/d)	No	2 months	5.6	↑	↑	NSC	NSC	—	—	↑	↑
			3 months	5.3	↑	↑	NSC	NSC	—	—	↑	↑
Svendsen et al. (2007), Norway	F&V intake (≥400 g/d veg & ≥300 g/d fruit)	No	3 months	6.0	↑ ‡	↑ ‡	↑ ‡	↑ ‡	NSC ‡	NSC ‡	—	—
Polidori et al. (2009), Germany	F&V intake (≥400 g/d)	No	1 month	NR†	↑	↑	NSC	↑	↑	NSC	—	↑
			2 months		↑	↑	NSC	↑	↑	NSC	—	↑
			3 months		↑	↑	NSC	↑	↑	NSC	—	↑
Rock et al. (2001), USA	F&V (8–10 servings/d)	No	6 months	2.8	↑	↑	↑	NSC	—	—	NSC	—
Bernstein et al. (2002), USA	F&V (≥5 servings/d)	No	6 months	2.2	↑	↑	↑	NSC	—	—	NSC	NSC
John et al. (2002)[1], UK	F&V (≥5 servings/d)	No	6 months	1.5	↑	↑	↑	NSC	↑	—	—	↑
Huxley et al. (2004)[2], UK	F&V (≥5 servings/d)	No	6 months	1.4	—	—	—	—	—	—	—	—
Cartmel et al. (2005)[1], USA	F&V (≥7 servings/d)	No	6 months	2.6	NSC	NSC	NSC	NSC	NSC	NSC	—	—
Takashashi et al. (2003)[1], Japan	↑ F&V intake to ↑ carotene & vitamin C intake	No	10 months	0.4	↑	NSC	—	—	—	—	—	NSC
Smith-Warner et al. (2000)[1], USA	F&V (≥8 servings/d)	No	1 year	4.6	↑	↑	↑	NSC	—	—	↑	—
Steptoe et al. (2003)[1], UK	1) ↑ F&V intake (NC)	No	1 year	1) 0.9	—	↑	—	—	—	—	—	NSC
	2) ↑ F&V intake (BC)			2) 1.5	—	↑	—	—	—	—	—	NSC
Djuric et al. (2006), USA	1) High F&V diet (9 servings)	No	1 year	1) 7.0 ‡	↑ ‡	↑ ‡	↑ ‡	NSC ‡	↑ ‡	NSC ‡	—	↑ ‡
	2) LF & high F&V diet			2) 6.8 ‡	↑ ‡	↑ ‡	↑ ‡	NSC ‡	↑ ‡	NSC ‡	—	↑ ‡

F&V = fruit and vegetables; **LF** = low fat; **NC** = Nutrition counselling; **BC** = Behavioral counselling;
↑ = significant increase in biomarker; ↓ = significant decrease in biomarker; — = not measured; **NR** = not reported; **NSC** = no significant change;
*Increase in intake calculated based on UK Department of Health guidance on portion sizes (1 serving = 80 g fruit/vegetables or 150 mL juice) - data given is reported increase in intake unless otherwise stated;
[1]Study reported between group statistics.
[2] Huxley et al. (2004) measured quercetin, kaempferol, and isorhamnetin (see text for details);
#Information not available from author;
**Approximate increase only (food intake not recorded throughout intervention);
†Unable to calculate increase in F&V intake from data collected;
‡Data obtained by personal communication with the authors;
§After 4 weeks on the intervention diet.

Table 3a Change in potential biomarkers of fruit and vegetable intake in response to increased consumption of mixed fruit and/or vegetables: Food provision studies

Study	Intervention	Depletion Period	Intervention duration	Increase in F&V intake (servings/d)*	Biomarker response to intervention							
					α-carotene	β-carotene	β-cryptoxanthin	Lycopene	Lutein	Zeaxanthin	Lutein + zeaxanthin	Vitamin C
Singh et al. (1995), India	400 g F&V + legumes/d + LF, low-energy diet	No	1 week	4.5	—	—	—	—	—	—	—	↑
Brevik et al. (2004a)[2], Norway	5 servings F&V/d (750 g)	1 week – avoid F&V	2 weeks	9.4[a]	—	—	—	—	—	—	—	—
Brevik et al. (2004b)[1], Norway	5 servings F&V/d (750 g)	1 week – avoid F&V	2 weeks	9.4[a]	↑	↑	NSC	NSC	↑	NSC	—	—
Thompson et al. (2005b), USA	12.1 servings/d F&V	No	2 weeks	12.1[a]	↑ ‡	↑ ‡	↑ ‡	↑ ‡	↑ ‡	—	—	—
Thompson et al. (2006), USA	8–10 servings F&V/d (HBD)	No	2 weeks	1) 4.0	↑	NSC	NSC	NSC	—	—	—	—
	8–10 servings F&V/d (LBD)			2) 4.6	↓	NSC	↑	↑	—	—	—	—
Roberts et al. (2003), UK	5 servings F&V/d	No	3 weeks	3.6	↑	↑	↑	NSC	—	—	↑	↑
Moller et al. (2003), Denmark	600 g F&V/d	No	24 days	3.3	—	↑	—	—	—	—	—	NSC
Dragsted et al. (2004), Denmark	600 g F&V/d	2 weeks – ≤ 2-4	16 days	3.4	NR	↑ †	NR	↑ †	NR	NR	—	NSC **
		portions F&V	25 days		NR	↑ †	NR	↑ †	NR	NR	—	NSC **
Thompson et al. (2005a)[1], USA	High F&V (9.2 servings/d)	No	2 weeks	4.9	↑	↑	↑	↑	↑	—	—	—
			4 weeks		↑	↑	↑	↑	↑	—	—	—
Broekmans et al. (2000), The Netherlands	High F&V (500 g/d) 200 mL/d fruit juice	No	4 weeks	4.5	↑	NSC	↑	↓	NSC	NSC	—	↑
Briviba et al. (2008)[1], Germany	5 servings F&V/d	4 weeks - ≤ 2	4 weeks	2.7	NSC	NSC	NSC	NSC	↑	↑	—	NSC
	8 servings F&V/d	servings F&V/d		5.9	↑	↑	NSC	NSC	↑	↑	—	NSC
van het Hof et al. (1999)[1], The Netherlands	High vegetable diet (490 g/d)	No	4 weeks	6.1	↑	↑	↑	↓	↑	↑	—	↑
Erlund et al. (2002)[3], Finland	High F&V	No	5 weeks	NR¥	—	—	—	—	—	—	—	—
McCall et al. (2009),	1) 3 servings F&V/d	4 weeks – 1	8 weeks	2.1	NSC	NSC	↑	NSC	↑	↑	—	↑
NI	2) 6 servings F&V/d	portion F&V/d		4.5	NSC	NSC	↑	NSC	↑	↑	—	↑
Franciose et al. (2006), USA	Diet rich in F&V	No	12 weeks	4.8#	NSC	NSC	↑	↑	↑	NSC	NSC	↑
Howe et al. (2009), USA	8 servings veg/d 2-3 servings fruit/d	No	12 weeks	3.4§	↑	↑	—	—	—	—	—	—
Bowen et al. (1993), USA	5-6 servings F&V/d	No	24 weeks	r	↑	↑	↑	↑	↑	—	—	—
				nr	↑	↑	↑	↑	NSC	—	—	—

F&V = fruit and vegetables; **LF** = low fat; **HBD** = High botanical diversity; **LBD** = Low botanical diversity;
↑ = significant increase in biomarker; ↓ = significant decrease in biomarker; — = not measured; **NR** = not reported; **NSC** = no significant change; **r** = responders; **nr** = non-responders

*Increase in intake calculated based on UK Department of Health guidance on portion sizes (1 serving = 80 g fruit/vegetables or 150 mL juice) - data given is reported increase in intake unless otherwise stated;

[a]Recommended increase in fruit or vegetable;

[1]Study reported between group statistics.

[2]Brevik et al. (2004a) measured quercetin and several other flavonoids (phloretin, eriodictyol, naringenin, hesperetin, kaempferol, isorhamnetin, and tamariexetin) (see text for details)

[3]Erlund et al. (2002) measured quercetin, hesperetin, and naringenin (See text for details)

†Data obtained through personal communication with the author.

#Intake of fruit and vegetables during study - baseline intake was not recorded and therefore an increase could not be calculated.

**Data provided in graph format but could not obtain raw data from author.

‡Estimated from Figure 1b, Thompson et al. (2005b) *J. Agric. Food Chem.* **53**: 6126–32.

¥Data not reported in paper and unavailable from author.

§ Data obtained from Table 3, Tanumihardjo et al. (2009) *Exp. Biol. Med.* **234**: 542–552 and through personal communication with the author.

Table 3b Change in potential biomarkers of fruit and vegetable intake in response to increased consumption of mixed fruit and/or vegetables: Food provision studies

Study	Intervention	Depletion Period	Intervention duration	Increase in F&V intake (servings/d*)	Biomarker response to intervention							
					α-carotene	β-carotene	β-cryptoxanthin	Lycopene	Lutein	Zeaxanthin	Lutein + zeaxanthin	Vitamin C
Chopra et al. (2000), UK	1) GW (300-400 g F&V/d)	1 week – avoid carotenoid containing veg.	1 week	3.8–5.0[a]	—	1) ↑	—	1) NSC	1) ↑	—	—	1) ↑
	2) RW (300-400 g F&V/d)				—	2) NSC	—	2) ↑	2) NSC	—	—	2) ↑
Martini et al. (1995)[1], USA	1) Carotenoid diet (165 g carrot coins, 125 g carrot puree and 250 g spinach)	No	10 days	1) 4.8	↑	↑	NSC	NSC	—	—	↑	—
	2) Cruciferous diet (390 g broccoli and 300 g cauliflower)			2) 6.6	NSC	NSC	NSC	NSC	—	—	↑	—
Tuekpe et al. (2006)[2], Japan	350 g/d Okinawan vegetable	No	2 weeks	3.6[a]	—	—	—	—	—	—	—	—
Rock et al. (1998), USA	1) Raw carrot (54.9 g) or spinach (39 g)	No	4 weeks	1) 0.7&0.5[a]	↑	NSC	NR	NR	NR	NR	—	—
	2) Processed carrot (113 g) or spinach (113 g)			2) 1.4&1.4[a]	↑	↑	NR	NR	NR	NR	—	—
Lin et al. (2007), Taiwan	1.5 servings stir-fried vegetables and 1 serving fruit	1 week – avoid carotenoid rich foods	1 week	2.5[a]	NR	↑ (m)	↑ (m)	↑ (m)	—	—	NSC (m)	—
			4 weeks		NR	↑ (w)	↑ (w)	↑ (w)	—	—	↑ (w)	—
					NR	↑ (m)	↑ (m)	↑ (m)	—	—	↑(w)	—
					NR	↑ (w)	↑ (w)	↑ (w)	—	—	↑(w)	—
Nielsen et al. (2002)[3], Finland	P2 or M2	No	6 weeks	P2) 4.7	—	—	—	—	—	—	—	—
				M2) 5.1	—	—	—	—	—	—	—	—
Freese et al. (2004)[§], Finland	P2 or M2	No	6 weeks	P2) 4.7	↑	↑	↑	NSC	↑	—	—	↑
				M2) 5.1	↑	↑	↑	↓	↑	—	—	↑
Erlund et al. (2006) [4], Finland	P2 or M2	No	6 weeks	P2) 4.7	—	—	—	—	—	—	—	—
				M2) 5.1	—	—	—	—	—	—	—	—
Khan et al. (2007)[1], Vietnam	1) Leafy vegetables 6 days/week	No	10 weeks	NR[¥]	↑	↓	↓	—	NSC	↓	—	—
	2) Mango or papaya 6 days/week				↑	↑	↑	—	↓	NSC	—	—
Sanchez-Moreno et al. (2004), Spain	Gazpacho soup (250 mL & 250 mL/d)	—	No	1 week	3.3[a]	—	—	—	—	—	—	—	↑
				2 weeks		—	—	—	—	—	—	—	↑
Sanchez-Moreno et al. (2006), Spain	Gazpacho soup (250 mL & 250 mL/d)	—	No	1 week	3.3[a]	—	—	—	—	—	—	—	↑
				2 weeks		—	—	—	—	—	—	—	↑
Abbey et al.(1995), Australia	250 mL orange juice & 300 mL carrot juice	Orange juice & carrot juice	3 weeks – avoid fruit juice	3 weeks	3.7[a]	—	↑	—	—	—	—	—	↑
Young et al. (1999), Denmark	1) 750 mL juice 2) 1000 mL juice 3) 1500 mL juice	Blackcurrant juice & apple juice (1:1)	No	1 week	1) 5.0[a]	—	—	—	—	—	—	—	NSC
					2) 6.7[a]	—	—	—	—	—	—	—	NSC
					3) 10.0[a]	—	—	—	—	—	—	—	↑
Thurmann et al. (2002), Germany	1) 250 mL juice 2) 750 mL juice	80% fruit juices & 20% carrot juice	No	6 weeks	1) 1.7[a]	↑	NSC	NSC	NSC	↑	NSC	—	—
					2) 5.0[a]	↑	↑	NSC	NSC	NSC	NSC	—	—
Wilms et al. (2005)[2], The Netherlands	1liter juice/d	Blueberry juice & apple juice (1:1)	5 days – avoid flavonoid rich foods	2 weeks 4 weeks	6.7[a]	—	—	—	—	—	—	—	—

						—	—	—	—	—	—	—	—
Paterson et al. (2006), UK	500 mL soup & 300 mL juice/100 mL shot/d	Juice: purple carrot, apple & strawberry Shot: 1) Orange, banana, carrot 2) Apple, carrot, strawberry 3) Carrot, banana, cherry	No	4 weeks	4.1	↑	↑	NSC	↑	—	—	↑	NSC
Weisel et al. (2006), Germany	700 mL mixed fruit juice/d	Red grape (57%) Blackberry (18%) Sour cherry (9%) Blackcurrant (9%) Chokeberry (7%)	No	4 weeks	4.7[a]	NSC	NSC	NSC	NSC	NSC	NSC	—	—
Wilms et al. (2007)[4], The Netherlands	1 liter juice/d	Blueberry juice & apple juice (1:1)	5 days – avoid flavonoid rich foods	4 weeks	6.7[a]	—	—	—	—	—	—	—	↑
Erlund et al. (2008)[1], Finland	100 g bilberries and 50 g crushed lingonberries 100 g blackcurrant/ strawberry puree and chokeberry and raspberry juice 70 mLs (on alternate days)	Chokeberry (80%) juice and 20% raspberry juice	No	2 weeks	3.9[†]	—	—	—	—	—	—	—	↑
				5 weeks		—	—	—	—	—	—	—	↑
				8 weeks		—	—	—	—	—	—	—	↑

F&V = fruit and vegetables; **GW** = Green week - at least 200 g creamed spinach and 100 g mango puree/d in addition to green vegetables but refraining from red vegetables for that week; **RW** = Red week - at least 200 g tomato puree and 100 g watermelon/d in addition to red vegetables but refraining from green, orange and yellow vegetables for that week; **P2** = Diet rich in veg, berries, apple and linoleic acid; **M2** = Diet rich in veg, berries, apple and oleic acid;

↑ = significant increase in biomarker; ↓ = significant decrease in biomarker; — = not measured; **NR** = not reported; **NSC** = no significant change;

(m) = men; **(w)** = women;

[*]Increase in intake calculated based on UK Department of Health guidance on portion sizes (1 serving = 80 g fruit/vegetables or 150 mL juice) - data given is reported increase in intake unless otherwise stated;

[a]Recommended increase in fruit or vegetable;

[1]Study reported between group statistics.

[2]Tuekpe et al. (2006) only measured urinary potassium and reported a significant increase from baseline.

[3]Nielsen et al. (2002) only measured flavonoids (12 dietary flavonoids) and reported a significant increase from baseline in the P2 and M2 diets.

[4]Erlund et al. (2002) only measured quercetin (see text for details)

[§]Biomarkers response in intervention group relative to baseline as reported in Table 5, Freese et al. (2002) *Am. J. Clin. Nutr.* 76: 950–60.

[¥]Not reported in the paper and not available from the author. Intervention was designed to provide 5.5 mg β-carotene/d in vegetable group and 4.8 mg β-carotene/d in the fruit group.

Food Provision Studies

Thirty-six papers were identified for inclusion in this section. Fruit and vegetables to be consumed were provided to participants. All but five of the studies in this section were conducted in healthy participants; the others focused on myocardial infarction patients (Singh et al., 1995), hypertensive patients (McCall et al., 2009), subjects at risk of cardiovascular disease (Erlund et al., 2008), subjects at risk for macular degeneration (Franciose et al., 2006), and lactating women (Khan et al., 2007).

The 17 studies in Table 3a, generally required participants to consume at least 5 portions of a variety of fruit and vegetables per day and most were less than 5 weeks duration (n = 13). In the 19 studies detailed in Table 3b, the study protocol was more prescriptive in terms of the specific fruits and/or vegetables that were to be consumed by participants, but did require consumption of more than one type of fruit and/or vegetable.

The number of biomarkers measured varied from one to eight, with 58% of studies (n = 21) measuring four or more biomarkers. Only one study (Weisel et al., 2006) out of 36 did not detect a significant increase in any of the biomarkers it measured. The responsiveness of the individual biomarkers was as follows: β-carotene increased significantly in 19 out of 24 (79%) studies, α-carotene in 16 out of 21 (76%) studies, vitamin C in 14 out of 18 (78%) studies, β-cryptoxanthin in 12 out of 20 (60%) studies, lycopene in eight out of 20 (40%) studies (but also significantly decreased in four out of 20 (20%) studies), lutein in 11 out of 16 (69%) studies, zeaxanthin in three out of 11 (27%) studies, and lutein/zeaxanthin in four out of five studies (80%). Urinary potassium was measured in two studies (Tuekpe et al., 2006; Erlund et al., 2008) but only increased significantly in one (50%) (Tuekpe et al., 2006; data not shown). Total carotenoids were measured in two studies (Paterson et al., 2006; Briviba et al., 2008), one of which (50%) (Briviba et al., 2008) reported significant increases. Quercetin increased significantly in five (Brevik et al., 2004a; Wilms et al., 2005; 2007; Erlund et al., 2006; 2008) out of seven (71%) studies (Young et al., 1999; Erlund et al., 2002; 2006; Brevik et al., 2004a; Wilms et al., 2005; 2007; Erlund et al., 2008; data not shown). Finally, in addition to quercetin, a number of different flavonoids (naringin, rutin, morin, phloretin, eriodictyol, naringenin, hesperetin, kaempferol, quercetin-3-O-Gal, quercetin-3-O-Glc, 5,7,8-trihydroxyflavone, isorhamnetin, and tamariexetin) were measured in three studies and increased significantly in all three (100%) (Erlund et al., 2002; Nielsen et al., 2002; Brevik et al., 2004a).

Two studies in Table 3a employed a dose-response design (Briviba et al., 2008; McCall et al., 2009), however, only one paper (McCall et al., 2009) reported linear trend data for biomarker response. McCall et al. (2009) reported a significant linear trend in the biomarker response (vitamin C, lutein, β-cryptoxanthin, and α-carotene) for one versus three versus six portions of fruit and vegetables per day; this indicated that a dose-response relationship existed between fruit and vegetable intake and these biomarkers in this eight week intervention in hypertensive volunteers.

Individual Fruit and Vegetable Studies

Thirty-three studies reporting the effect of individual varieties of fruit and vegetables on biomarkers of interest were identified by the search strategy. The intervention group data from these studies has been summarized in Tables 4 and 5 for vegetables and vegetable juices, and fruit and fruit juices, respectively. These studies were all carried out in healthy adults, with the exception of the studies by Singh et al. (1997) in hypertensive individuals and Burr et al. (2007) in pregnant women. In the majority of studies, the fruit and/or vegetable under investigation was provided to participants but consumed in a free-living situation, that is, not under the supervision of the research team. The only exceptions to this were two studies that supplied the fruit and/or vegetable under investigation but it was consumed under supervision (de Vries et al., 1998; Moon et al., 2000) and seven studies that supplied participants with their total dietary intake: this was consumed in a free-living situation in one study (Harats et al., 1998), partially supervised in four studies (Kim et al., 1988; Micozzi et al., 1992; Mangels et al., 1993; Castenmiller et al., 1999) and completely supervised in two studies (Haskell et al., 2004; Yeum et al., 1996), the latter of which was a residential study.

Vegetables and Vegetable Juices

As indicated in Table 4, change in biomarkers in response to increased intake of a specific vegetable was examined in 21 studies. The vegetables used in these studies included carrot (Kim et al., 1988; Micozzi et al., 1992; Torronen et al., 1996; Muller et al., 1999; Watzl et al., 1999; 2003; Astley et al., 2004; Molldrem et al., 2004), spinach (Castenmiller et al., 1999; Haskell et al., 2004; Kopsell and Lefsrud, 2006), broccoli (Micozzi et al., 1992; Mangels et al., 1993; Yeum et al., 1996; Granado et al., 2006), sprouts (Bogaards et al., 1994; Nijhoff et al., 1995; Gill et al., 2004; Hoelzl et al., 2008), watercress (Gill et al., 2007) and onions (de Vries et al., 1998; Moon et al., 2000).

The most commonly studied vegetable in this category was carrot, either whole or in the form of carrot juice. Increases in intake of this vegetable and juice ranged from 0.67-4.2 servings per day. This was accompanied by consistently significant increases in α- and β-carotene, with the exception of a yellow carrot intervention by Molldrem et al. (2004) which resulted in no significant change in β-carotene. Torronen et al. (1996) was the only study to directly compare carrots with carrot juice in terms of biomarker response. Participants in this study consumed either 1.5 servings of raw carrot or 0.67 servings of carrot juice per day for 6 weeks. Beta-carotene was the only biomarker measured; it increased significantly within both the raw carrot and carrot juice groups with no significant differences between the groups.

In the three spinach studies shown in Table 4, two measured α-carotene and reported a significant increase in association with increases in spinach intake of 0.25–1 serving per day for 3–8 weeks. Beta-carotene was measured in all 3 studies and increased significantly in two.

Table 4 Change in potential biomarkers of fruit and vegetable intake in response to increased consumption of individual varieties of vegetables or vegetable juices

					Biomarker response to intervention							
Study	Intervention	Depletion Period	Intervention duration	Increase in F&V intake (servings/d)*	α-carotene	β-carotene	β-cryptoxanthin	Lycopene	Lutein	Zeaxanthin	Lutein + zeaxanthin	Vitamin C
Kim et al. (1988), USA	16 oz carrot juice/d for 1 week and 8 oz carrot juice every other day for an additional week	No	1 week	3.0[a]	↑	↑	—	—	—	—	—	—
			2 weeks	1.5[a]	↑	↑	—	—	—	—	—	—
Muller et al. (1999), Germany	330 mL carrot juice/d	2 weeks – avoid carotenoid rich food	2 weeks	2.2[a]	↑	↑	NSC	NSC	—	—	NSC	—
Watzl et al. (1999), Germany	330 mL carrot juice/d	2 weeks – avoid carotenoid rich food	2 weeks	2.2[a]	—	↑	—	NSC	NSC	—	—	NSC
Watzl et al. (2003), Germany	330 mL carrot juice/d	2 weeks – avoid carotenoid rich food	2 weeks	2.2[a]	↑	↑	↓	NSC	NSC	NR	—	—
Molldrem et al. (2004)[1], USA	Yellow carrots	4 days – avoid high lutein foods	1 week	4.2	—	NSC	—	—	↑	—	—	—
Astley et al. (2004), UK	200 g minced carrots/d	—	3 weeks	2.5[a]	↑	↑	NSC	↑ ‡	NSC	NSC	—	NSC
Torronen et al. (1996), Finland	1) 120 g raw carrots/d	10 days – avoid carotenoid rich foods	3 weeks	1.5[a]	—	1) ↑	—	—	—	—	—	—
	2) 100 mL carrot juice/d		6 weeks	0.67[a]	—	2) NSC	—	—	—	—	—	—
						1) ↑	—	—	—	—	—	—
						2) ↑	—	—	—	—	—	—
Castenmiller et al. (1999)[1] The Netherlands	1) 20 g whole leaf spinach	3 weeks – avoid carotenoid and vitamin A rich foods	3 weeks	0.25[a]	↑ ‡	↑	NSC	↓ ‡	↑	NSC	—	—
	2) 20 g minced spinach			0.25[a]	↓ ‡	↑	NSC	↓ ‡	↓ ‡	NSC	—	—
	3) 20 g liquefied spinach			0.25[a]	↓ ‡	↑	NSC	↓ ‡	↓ ‡	NSC	—	—
	4) 20 g liquefied spinach with fiber			0.25[a]	↓ ‡	↑	NSC	↓ ‡	↓ ‡	NSC	—	—
Haskell et al. (2004), Bangladesh	1) Sweet potato (80 g) and corn oil capsule	3 days – avoid vitamin A foods	60 days	1.0[a]	NSC	↑	—	—	NSC	—	—	—
	2) Indian spinach (75 g) and corn oil capsule			0.94[a]	↑	↑	—	—	↑	—	—	—
Kopsell and Lefsrud (2006), USA	1) High-Lutein spinach (50 g/d for 5 days/wk)	No	12 wks	0.63[a]	—	NSC	—	—	↑	NSC	↑	—
	2) Low-Lutein spinach (50 g/d for 5 days/wk)			0.63[a]	—	NSC	—	—	NSC	NSC	NSC	—
Granado et al. (2006), Spain	200 g broccoli/d	No	1 week	2.5[a]	NSC	NSC	↓	↓	↑	↓	—	—
Yeum et al. (1996), USA	204.8 g broccoli/d for 5 days in addition to a high carotenoid diet (consumed for 15 days)	No	6 - 16 days	2.6[a]	↑**	↑**	↑**	↑**	↑**	↑**	—	—

(*Continued on next page*)

Table 4 Change in potential biomarkers of fruit and vegetable intake in response to increased consumption of individual varieties of vegetables or vegetable juices *(Continued)*

					Biomarker response to intervention							
Study	Intervention	Depletion Period	Intervention duration	Increase in F&V intake (servings/d)*	α-carotene	β-carotene	β-cryptoxanthin	Lycopene	Lutein	Zeaxanthin	Lutein + zeaxanthin	Vitamin C
Mangels et al. (1993), USA	1) Raw broccoli (91 g)	No	4 weeks	1.1[a]	—	—	—	—	—	—	—	↑
	2) Cooked broccoli (154 g)			1.9[a]	—	—	—	—	—	—	—	↑↑
Micozzi et al. (1992)[1], USA	1) Broccoli (300 g)	No	6 weeks	3.8[a]	↓	NSC	—	NSC	—	—	↑	—
	2) Carrots (272 g)			3.4[a]	↑	↑	—	NSC	—	—	↑	—
Hoelzl et al. (2008), Austria	300 g Brussels sprouts/d	5 days - ≤ 2000 g/d citrus fruits, fruit juice and onions	6 days	3.8[a]	—	—	—	—	—	—	—	↑
Nijhoff et al. (1995)[2], The Netherlands	300 g Brussels sprouts/d	No	1 week	3.75[a]	—	—	—	—	—	—	—	—
Bogaards et al. (1994)[3], The Netherlands	300 g Brussels sprouts/d	3 weeks – avoid glucosinolates-containing food	3 weeks	3.75[a]	—	—	—	—	—	—	—	—
Gill et al. (2004), Northern Ireland	113 g cruciferous and legume sprouts/d	No	2 weeks	1.4[a]	NSC	NSC	NSC	NSC	NSC	—	—	—
Gill et al. (2007), Northern Ireland	85 g raw watercress/d	No	8 weeks	1.06[a]	—	↑	—	—	↑	—	—	NSC
de Vries et al. (1998)[4], The Netherlands	1) 129 g fried onions/d for 3 days	4 days – avoid quercetin rich foods	1 week	1.6[a]	—	—	—	—	—	—	—	—
	2) 129 g fried onions/d for 3 days			1.6[a]	—	—	—	—	—	—	—	—
Moon et al. (2000)[5], Japan	260-360 g onion slices/d	No	1 week	3.25–4.5[a]	—	—	—	—	—	—	—	—

F&V = fruit and vegetables;

↑ = significant increase in biomarker; ↓ = significant decrease in biomarker; — = not measured; **NR** = not reported; **NSC** = no significant change;

* Increase in intake calculated based on UK Department of Health guidance on portion sizes (1 serving = 80 g fruit/vegetables or 150 mL juice) - data given is reported increase in intake unless otherwise stated;

[a] Recommended increase in fruit or vegetable;

[1] Study reported between group statistics;

[2] Nijhoff et al. (1995) only measured α- and π- class glutathione S-transferase in plasma and urine (see text for details);

[3] Bogaards et al. (1994) only measured α-class glutathione S-transferase in plasma (see text for details);

[4] de Vries et al. (1998) only measured quercetin and kaempferol (see text for details);

[5] Moon et al. (2000) only measured quercetin (see text for details);

‡ Data obtained by personal communication with author;

** Estimated from Figures 2, 4, and 6, Yeum et al. (1996) *Am. J. Clin. Nutr*. **64**: 594–602.

Table 5 Change in potential biomarkers of fruit and vegetable intake in response to increased consumption of individual varieties of fruit or fruit juices

					Biomarker response to intervention							
Study	Intervention	Depletion Period	Intervention duration	Increase in F&V intake (portions/d)*	α-carotene	β-carotene	β-cryptoxanthin	Lycopene	Lutein	Zeaxanthin	Lutein + zeaxanthin	Vitamin C
Harats et al. (1998), Israel	500 mg/d vitamin C from oranges for 2 months	4 weeks - ≤ 50 mg/d vitamin C	1 month 3 months	6.0[a,**]	—	—	—	—	—	—	—	↓ ↑
Johnston et al. (2003), USA	1) 8 oz OJ	2 weeks – avoid vitamin C rich foods	2 weeks	1.5[a]	—	—	—	—	—	—	—	NSC
	2) 16 oz OJ			3.0[a]	—	—	—	—	—	—	—	↑
Sanchez-Moreno et al. (2003), Spain	2 × 250 mL OJ/d	No	1 week	3.33[a]	—	—	—	—	—	—	—	↑
			2 weeks		—	—	—	—	—	—	—	↑
Astley et al. (2004), UK	Tinned mandarin oranges (298 g/d)	No	3 weeks	3.73[a]	NSC	NSC	↑	NSC	NSC	NSC	—	↑
Mangels et al. (1993), USA	1) orange segments (150 g)	No	4 weeks	1.88[a]	—	—	—	—	—	—	—	↑
	2) orange juice (236 mL)			1.57[a]	—	—	—	—	—	—	—	↑
Burr et al. (2007), UK	1) Advice - ↑ fruit and fruit juice	No	8 months	NR[¥]	—	1) NSC	—	—	—	—	—	—
	2) Voucher - OJ purchase				—	2) ↑	—	—	—	—	—	—
Marniemi et al. (2000), Finland	100 g berries/d	No	2 weeks	1.25[a]	—	NSC	—	—	—	—	—	↑
			4 weeks		—	NSC	—	—	—	—	—	↑
			8 weeks		—	NSC	—	—	—	—	—	↑
Erlund et al. (2006), Finland	100 g berries/d	No	2 weeks	1.25[a]	—	—	—	—	—	—	—	—
			4 weeks		—	—	—	—	—	—	—	—
			8 weeks		—	—	—	—	—	—	—	—
Singh et al. (1997), India	347 g guava/d	No	24 weeks	4.34[a]	—	—	—	—	—	—	—	↑
Freedman et al. (2001), USA	Purple grape juice (7 mL/kg/d)	No	2 weeks	3.3[a] ‡	—	—	—	—	—	—	—	NSC
Edwards et al. (2003), USA	1) ~ 413 mLs watermelon juice/d	2 weeks avoid lycopene rich foods	1 week	2.75[a]	—	↑	—	↑	—	—	—	—
	2) ~ 826 mLs watermelon juice/d		2 weeks		—	↑	—	↑	—	—	—	—
			3 weeks		—	↑	—	↑	—	—	—	—
			1 weeks	5.5[a]	—	↑	—	↑	—	—	—	—
			2 weeks		—	↑	—	↑	—	—	—	—
			3 weeks		—	↑	—	↑	—	—	—	—
Duttaroy and Jorgensen (2004), Norway	1) Two kiwi/d	No	4 weeks	1.0[a]	—	—	—	—	—	—	—	↑
	2) Three kiwi/d			1.5[a]	—	—	—	—	—	—	—	↑
Tesoriere et al. (2004), Italy	500 g cactus pear pulp	No	2 weeks	6.25[a]	—	—	—	—	—	—	—	↑
Duthie et al. (2006), UK	3 × 250 mL cranberry juice/d	No	2 weeks	5[a]	—	—	—	—	—	—	—	↑

F&V = fruit and vegetables; **OJ** = orange juice

↑ = significant increase in biomarker; ↓ = significant decrease in biomarker; — = not measured; **NR** = not reported; **NSC** = no significant change;

*Increase in intake calculated based on UK Department of Health guidance on portion sizes (1 portion = 80 g fruit/vegetables or 150 mL juice) - data given is reported increase in intake unless otherwise stated;

[a]Recommended increase in fruit or vegetable;

[1]Erlund et al. (2006) only measured quercetin (see text for details)

[¥]Unable to calculate increase in F&V intake as subjects were asked about frequency of consumption of different types of F &V each day and not the amount eaten each day;

‡Based on average weight of a man (70 kg)

**Increase estimated by converting quantity of oranges needed to give 500 mg vitamin C into portions using food composition tables (McCance and Widdowson (2002) The Composition of Foods Sixth summary edition. Royal Society of Chemistry, Cambridge).

In the four broccoli intervention studies detailed in Table 4, intake increased by 1.1-3.8 servings per day and biomarker response was mixed. Alpha-carotene, β-carotene, and lycopene increased significantly in one (Yeum et al., 1996) out of the three studies that measured it; however, in this study by Yeum et al. (1996), the broccoli was consumed in addition to a high carotenoid diet thus explaining these increases. Lutein increased in both studies that measured it (Yeum et al., 1996; Granado et al., 2006); beta-crytoxanthin concentrations were also measured in these two studies and increased significantly in one (Yeum et al., 1996). Lutein/zeaxanthin concentrations were measured in one study (Micozzi et al., 1992) and increased significantly. Vitamin C was measured in one study (Mangels et al., 1993) and increased significantly.

Five studies examined biomarker response to cruciferous and legume sprouts and watercress. In the sprout studies, healthy adults consumed either 113 g (approximately 1.4 servings) (Gill et al., 2004) or 300 g (3.8 servings) (Bogaards et al., 1994; Nijhoff et al., 1994; Hoelzl et al., 2008) of Brussels sprouts per day for 6 days to three weeks. The study by Gill et al. (2004) measured 5 biomarkers of intake (α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein) but did not observe any significant changes in post-intervention concentrations. On the other hand, vitamin C was the only biomarker measured by Hoelzl et al. (2008) and 300 g of Brussels sprouts resulted in a significant increase in this biomarker. Glutathione S-transferase class-α was measured in plasma in one study (Bogaards et al., 1994) and class-α and -π were measured in plasma and urine in another study (Nijhoff et al., 1995). Class-α increased significantly in these two studies but class-π remained unchanged (data not shown). Gill et al. (2007) also report results for another cruciferous vegetable, watercress (85 g watercress, n = 60 healthy adults for 8 weeks). Significant increases in lutein and β-carotene were observed, while the vitamin C status did not change.

Onion consumption was investigated on two occasions (de Vries et al., 1998; Moon et al., 2000); increases in intake equated to 1.6 and 4.5 servings per day respectively for one week. Both of these studies resulted in significant increases in quercetin, and de Vries et al. (1998) also reported a significant increase in the flavonol kaempferol.

In summary, 21 studies focusing on individual varieties of vegetables and vegetable juices were included in this section, the number of biomarkers measured varied from one to eight; 33% (n = 7) of the studies measured one biomarker, 33% (n = 7) measured two to four biomarkers, and 33% (n = 7) measured five to eight biomarkers. Only one study out of 21 did not detect a significant increase in measured biomarkers in response to increased vegetable intake; the study by Gill et al. (2004) measured 5 biomarkers but, crucially, did not measure vitamin C.

Fruit and Fruit Juices

Fourteen studies examined change in biomarkers in response to increased intake of a specific type of fruit or fruit juice as summarized in Table 5. The type of fruit used in these studies included oranges (Mangels et al., 1993; Harats et al., 1998; Johnston et al., 2003; Sanchez-Moreno et al., 2003; Astley et al., 2004; Burr et al., 2007), berries (Marniemi et al., 2000; Erlund et al., 2006), guava (Singh et al., 1997), grape juice (Freedman et al., 2001), watermelon juice (Edwards et al., 2003), cactus pear (Tesoriere et al., 2004), kiwi (Duttaroy and Jorgensen, 2004), and cranberry juice (Duthie et al., 2006).

Nearly half of the studies in this category investigated orange segment or orange juice consumption (n = 6). Vitamin C was measured in five out of the six orange intervention studies, and significantly increased in all five studies. Beta-carotene also increased significantly in one of the two studies that measured it, and β-cryptoxanthin increased significantly in the only orange intervention study that measured it (Astley et al., 2004; this study measured a panel of carotenoids but β-cryptoxanthin was the only biomarker to increase significantly).

The two berry papers (Marniemi et al., 2000; Erlund et al., 2006) had similar protocols; increased intake of approximately 1.25 servings of berries per day was associated with significant increases in quercetin (Erlund et al., 2006, only reported results for quercetin) and vitamin C, but not β-carotene (Marniemi et al., 2000).

Similarly to the orange and berry studies, the cactus pear (Tesoriere et al., 2004), kiwi fruit (Duttaroy and Jorgensen, 2004), purple grape juice (Freedman et al., 2001), watermelon juice (Edwards et al., 2003), cranberry juice (Duthie et al., 2006), and guava (Singh et al., 1997) studies only measured a few select biomarkers; the grape juice study was the only study of these that did not report a significant increase in their biomarker of interest (vitamin C).

In summary, 14 studies focusing on individual varieties of fruit and fruit juices were included in this section, the number of biomarkers measured varied from one to eight, with 79% (n = 11) of studies only measuring one biomarker (in nine out of these 11 studies this biomarker was vitamin C). Only one study (Freedman et al., 2001; purple grape juice study) out of 14 did not detect a significant increase in biomarkers in response to the intervention; however, only vitamin C was measured. In terms of individual biomarker responses, vitamin C was by far the most commonly measured biomarker in this category and increased significantly in 10 out of 11 (91%) studies. The other individual biomarkers were only measured in a small number of studies.

DISCUSSION

General Fruit and Vegetable Intake—Carotenoids as Biomarkers

Alpha- and β-carotene, lutein, and lutein/zeaxanthin increased significantly in 74%, 76%, 71%, and 69% of the general fruit and vegetable studies that measured them, respectively. Lycopene decreased significantly in four studies (van het Hof et al., 1999; Broekmans et al., 2000; Freese et al., 2004; Thompson

et al., 2006); however, this is likely to have been owing to the removal of lycopene rich fruits and vegetables from the diets of participants in favor of other fruits and vegetables (Broekmans et al., 2000).

There are a number of factors that will affect the response of carotenoids to increased fruit and vegetable consumption and, thus, may affect their ability to act as reliable biomarkers of fruit and vegetable consumption. The composition of the diet eaten alongside fruit and vegetables is known to influence carotenoid response, for example, concurrent ingestion of carotene free plant materials (Huang et al., 2000) can decrease bioavailability of carotene from other sources, and consumption with a fat source (Brown et al., 2004; Unlu et al., 2005) can enhance the absorption of carotenoids as they are fat-soluble nutrients. However, it does appear, at least for intervention studies, that a panel of carotenoids will reflect increased consumption of mixed fruit and vegetables. Most studies, to date, have reported individual carotenoids; however, total carotenoids may also represent a potentially useful biomarker approach; they were measured in 13 studies in the "whole diet" and "mixed fruit and vegetable" sections and increased significantly in 7 of these studies (54%).

General Fruit and Vegetable Interventions – Vitamin C as a Biomarker

Vitamin C increased significantly in 21 out of 29 (72%) studies in the "whole diet" and "mixed fruit and vegetable" sections. Its use as a biomarker of fruit and vegetable intake may, however, be limited in already well nourished populations as the relationship between vitamin C intake and plasma concentration is linear only up to a certain threshold (Padayatty and Levine, 2008). Indeed, in the eight studies that did not show a significant increase in vitamin C (Bernstein et al., 2002; Moller et al., 2003; Nelson et al., 2003; Steptoe et al., 2003; Takashashi et al., 2003; Dragsted et al., 2004; Paterson et al., 2006; Briviba et al., 2008), baseline vitamin C status was approximately 60 μmol/L or higher; evidence indicates that plasma concentrations may be beginning to plateau at these levels and therefore may not be significantly augmented in response to a dietary intervention (Levine et al., 2001).

Another weakness of using vitamin C as a biomarker of fruit and vegetable consumption is that the assessment of vitamin C in biological samples requires precise sample handling and stabilization before storage. Vitamin C is labile and may degrade during long periods of storage. These factors do not lend themselves to large-scale epidemiological studies, although analysis should still be possible in smaller intervention studies (Jenab et al., 2009).

A cross-comparison of studies that assessed vitamin C status in the "whole diet" and "mixed fruit and vegetable" sections is made more difficult by the fact that some countries, such as the United States, include potatoes in their "fruit and vegetable" food group, whereas others classify this food as a carbohydrate or starchy food with breads, cereals, and potatoes, such as in the UK. Potatoes are not a rich source of vitamin C but can make an important contribution to vitamin C intakes owing to the quantity in which they are consumed. Five relevant US-based studies in the two afore-mentioned categories measured vitamin C status; two noted no significant change and three noted a significant increase in status. Three UK-based studies assessed vitamin C status; one noted no significant change and two noted a significant increase in status. Hence, no major disparity that was possibly attributable to increased potato consumption was obvious.

General Fruit and Vegetable Interventions—Other Potential Biomarkers

Relatively few mixed fruit and vegetable studies have assessed other potential single biomarkers of fruit and vegetable consumption, such as quercetin or potassium, and therefore further work must establish whether these nutrients do respond to a mixed fruit and vegetable intervention. A potentially useful approach, similar to the total carotenoid approach detailed above, is the use of an estimate of overall flavonoid status. Several observational and intervention studies have attempted to deal with the large number of flavonoids found in fruit and vegetables by simply summing total flavonoid excretion in urine (Nielsen et al., 2002; Krogholm et al., 2004; Mennen et al., 2006), with a varying degree of success [exemplar correlations: correlation coefficients of $r = 0.38$, fruit and fruit juice consumption (Mennen et al., 2006); $r = 0.35$, habitual fruit and vegetable consumption (Nielsen et al., 2002); and $r = 0.86$, controlled fruit and vegetable intervention, 24 hour urine (Krogholm et al., 2004)]. Mennen et al. (2006) suggest that adding more polyphenols to the comparison might improve the accuracy of the biomarker to take into account the diversity of contributing sources. However, no attempt has ever been made to examine blood levels of flavonoids or other polyphenols in this way. Although, in the context of a randomized-controlled fruit and vegetable trial, background diet should remain stable during the intervention period, the possibility remains that individuals may change other dietary habits and modify their intake of other flavonoid-rich foods (such as tea, coffee, cocoa, wine) which may present one possible limitation of this approach, and the use of flavonoids as biomarkers of fruit and vegetable consumption in general. Further research is, however, warranted to explore such confounding factors. Furthermore, no attempt has been made to model in more detail the association between total fruit and vegetable consumption and a panel of these potential flavonoid blood markers alongside vitamin C and carotenoids. Such an approach that takes into account the diversity of bioactive compounds found in fruit and vegetables, along with the consumption of a range of fruit and vegetables in a normal diet, may well be useful.

Biomarkers of Individual Fruit and Vegetables

In general, studies examining the effects of increased consumption of individual types of fruit and vegetables demonstrated significant increases in several biomarkers of interest

across studies from several different countries, of varying duration (1–24 weeks) and employing different levels of control ranging from provision of key foods with consumption in a free-living situation, through to supplying the total diet which was then consumed under close supervision.

Some general observations can be made regarding the results from the vegetable and vegetable juice studies, although some are based on a very small number of studies and require more extensive investigation. Quercetin was a responsive biomarker of increased onion consumption. Several biomarkers were responsive to increased broccoli consumption notably lutein, zeaxanthin and β-cryptoxanthin. Similarly, lutein, lycopene, and α- and β-carotene were all responsive to increased spinach consumption. The carotenoids α- and β-carotene were particularly responsive biomarkers of carrot consumption, and vitamin C, and glutathione S-transferase may be good biomarkers of sprout or cruciferous vegetable consumption, although this cannot be stated with any certainty based on data presented in this review. These results highlight that there are few vegetables for which it would be sufficient to just measure one biomarker and, as for the overall mixed fruit and vegetable interventions, measuring a panel of biomarkers is likely to yield the most meaningful results.

For the fruit and fruit juice studies, vitamin C was the most commonly measured biomarker in the fruit and fruit juice studies (measured in 11 out of 14 studies) and it was responsive in 10 out of those 11 studies indicating its usefulness as a biomarker in fruit-based interventions. Unlike the other categories, most studies in this section did not measure a panel of biomarkers. There is, therefore, scope for further research to examine other potential biomarkers of fruit intake, for example β-cryptoxanthin increased significantly in the only study in this category that measured a broad panel of biomarkers (Astley et al., 2004).

Further Considerations for Biomarker Development and Potential Weaknesses of the Systematic Review

There are many extrinsic and intrinsic factors that can ultimately influence biomarker response. The baseline nutrient status of the population will be important, as nutrient absorption can be greater when baseline levels are low (Jacques et al., 1987; Sanchez-Moreno et al., 2003). Some studies in this review employed a depletion period before commencing the intervention which may have increased the likelihood of observing a significant change in the relevant biomarkers. Indeed, all of the studies that incorporated a depletion period into their study design did report significant increases in at least one biomarker. Responsiveness of biomarkers may be altered by lifestyle and other factors, such as age, smoking behavior, physical activity, and the presence of low-grade inflammation. Such factors may have affected the response of the biomarkers to intervention in the studies summarized here, but the studies are too heterogeneous in terms of design, level of control of overall dietary intake, duration, and the nature of the control group to allow exploration of these issues. The level of nutrients in food will directly affect biomarker response and may vary by individual variety of fruit or vegetable (Heinonen, 1990; Kurilich and Juvik, 1999), production conditions (Kopsell and Lefsrud, 2006), and processing, storage, and cooking methods, particularly for vitamin C (Hussein and El-Tohamy, 1990; Castenmiller et al., 1999). It has been assumed that appropriate advice was given to participants or that appropriate storage and cooking methods were used by researchers; however, this may well have been inconsistent between studies.

In this review, we have examined biomarker response in comparison with reported change (or recommended change if data on reported change was unavailable) in fruit and vegetable intake, but this reported change is subject to errors, which can have a major impact on its accuracy. It is possible that study participants reported compliance with a dietary intervention through diet records, but were in fact not complying with the intervention, which may have led to a lack of biomarker response in some studies. Furthermore, it has been assumed in this review that the laboratory methods used in these studies were sensitive, specific, reproducible, precise and accurate, and subject to adequate quality assurance. A major difficulty when comparing biomarker responses across studies relates to problems with assay standardization. Not all researchers give details of their standardization procedures within their manuscripts and there is no international standardization of vitamin C or carotenoid measurement, although a number of quality assurance schemes exist and NIST reference materials are available for both vitamin C and carotenoids. This lack of standardization means that it cannot be assumed that a significant response in a biomarker in one laboratory would be detected in another laboratory and, again, some studies may have failed to detect significant increases owing to problems with their laboratory procedures.

In terms of satisfying the criteria for a good biomarker of dietary intake, using plasma or serum satisfies the criterion that biomarker assessment should be non-invasive or minimally invasive for participants. The methods available to measure these biomarkers are, for the most part, reproducible (bearing in mind the caveats discussed above), and are relatively straightforward for most laboratories involved in nutritional research. A good biomarker should also be able to discriminate between differences in intake; results of this review suggest that a panel of biomarkers currently exists that is responsive to even small changes in fruit and vegetable intake. Only one study to date provides within-study dose-response statistics (McCall et al., 2009), and clearly more studies of this nature will help to elucidate the linearity of response for specific biomarkers and, therefore, provide a satisfactory answer as to the responsiveness of these biomarkers.

CONCLUSION

In general, fruit and vegetable intervention studies of different type, duration, design, and intensity demonstrated

significant increases in several biomarkers of intake. The data presented indicates that a panel of biomarkers (notably α- and β-carotene, vitamin C, lutein, zeaxanthin, and β-cryptoxanthin) should be measured as indicators of compliance in fruit and vegetable intervention trials. Given the diverse range of bioactive compounds in fruit and vegetables, and the many intrinsic and extrinsic factors that can affect biomarker response, it is rarely possible to rely on assessment of a single biomarker as an indicator of dietary change in human intervention studies. The one possible exception to this is "fruit only" intervention studies where assessment of vitamin C alone may suffice. Further research should continue to explore more novel biomarker approaches.

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